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DIVISIONAL

APPLICATION

for

UNITED STATES LETTERS PATENT

on

METHOD FOR DETECTION OF BIOLOGICAL FACTORS IN EPIDERMIS

by

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Sheets of Drawings: **Two (2)**
Docket No.: **DERM1100-6**

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METHOD FOR DETECTION OF BIOLOGICAL FACTORS IN EPIDERMIS

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority under 35 U.S.C. § 120 of United States Application Serial No. 09/375,609, filed August 17, 1999, now pending, which claims priority under 35 U.S.C. § 119(e) of Provisional Application Serial No. 60/097,025, filed August 18, 1998, each of which is incorporated herein by reference in its entirety

FIELD OF THE INVENTION

[0002] This invention relates to method of detecting biological factors in epidermis, wherein the biological factor may be a polynucleotide or polypeptide encoded by the polynucleotide or a lipid.

BACKGROUND OF THE INVENTION

[0003] Cells and tissues are influenced by endogenous and exogenous agents and respond with a cascade of biological activities to mediate a response to an agent. For example, the skin is the site of many dermatological reactions that result from exposure of the skin to exogenous agents. The skin also is the most accessible organ in the body. Thus, the skin lends itself to access for determination of protein reactions, as well as, the gene(s) and gene products that are associated with or give rise to a particular reaction.

[0004] The epidermis is the outermost layer of the skin. This layer contains four major cell types. The most prevalent cell in the epidermis is the keratinocyte in various stages of differentiation. The epidermis maintains its pool of keratinocytes by mitosis of these cells in the basal cell layer, the lowest layer of the epidermis. By contrast, the upper most covering layer of the epidermis is the stratum corneum that, in normal skin, does not contain nucleated cells. Keratinocytes produce a number of cytokines including interleukin (IL) IL-I, IL-3, IL-4, IL-6, IL-7, IL-8, IL-l0, IL-12 and granulocyte macrophage colony stimulating factor (GM-CSF) Kupper, M., 1993. *Am. J. Dermatopathol.* 11:69-73). Above the basal cell layer, resides the Langerhans cell, an immune competent cell of bone marrow origin. The Langerhans cell has

features of macrophage as well as T cells and is thought to be responsible for initiating a series of events that lead to immune reactions in the skin such as a contact dermatitis. The melanocyte is the pigment producing cell of the skin. This cell also usually resides in the deeper layers of the epidermis. The fourth cell in the epidermis is the Merkel cell.

[0005] Immediately below the epidermis, resides the dermal layer which contains mainly fibroblasts, lymphocytes, mast cells, endothelial cells and nerve endings. Fibroblasts are the main cell type that deposit extracellular matrix material and structural proteins of the skin, such as collagen. The endothelial cells coat the lumina of the dermal capillaries and mast cells contain histamine that can be liberated in inflammatory responses of the skin. Inflammation of the skin may result from a broad array of external agents applied to the skin. Classes of contact dermatitis include irritant, allergic, photoallergic and phototoxic and subclinical mechanisms. Clinically, the reactions are virtually identical with the appearance of an eczematous process typified by erythema, edema and vesiculation (Hoefakker *et al.* 1995. *Contact Dermat.* 33:258-266; Krasteva, M. 1993. *Int. J. Dermatol.* 32:547-560). Contact uricaria is an additional potential response to skin application of various agents that differs in the immediate appearance of a wheal upon skin contact. Categorizing the mechanism of the contact reaction is of importance to patients. This stems from the immunologic consequences of an allergic or immune response leading to increasingly severe inflammation of the skin with re-exposure after sensitization. For example, characterizing the type of inflammatory response to exposure of an agent can provide both patients and manufacturers the ability to purchase and redesign products to avoid future inflammatory reactions.

[0006] The frequent and historical occurrence of contact dermatitis has provided the impetus for implementation of human skin testing for all new topical drugs or cosmaceuticals. A well defined arsenal to skin safety tests is now required to be conducted before any product destined to contact the skin can be put on the market in many countries. Predictive skin patch tests conducted with the product and its constituents have been the mainstay of this testing procedure. Since the inception of this predictive skin patch testing, a major deficiency has been the inability to clearly differentiate an irritant contact dermatitis (ICD) from an allergic contact dermatitis (ACD). Furthermore, the patch test is simply not sufficient for quantitatively measuring the